Temperature acclimation before shipping and light acclimation after shipping improved the performance of *Phalaenopsis* after prolonged dark shipping

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Abstract

Mature, ready-to-bloom *Phalaenopsis* plants are often shipped by sea, where they experience total darkness for a few weeks in cool air, in stark contrast to warm growing conditions in the greenhouse. After prolonged dark shipment, the plants experience a drastic change from total darkness to lighted conditions. The aim of this study was to investigate the effects of lower-temperature acclimation before shipping and light acclimation after dark storage on the quality and photosynthetic status of *Phalaenopsis* plants. Temperature acclimation at 25/20 °C day/night temperatures for 10 d before shipment reduced the severity of chilling injury in mature *Phalaenopsis* plants in a cool and dark environment. The increased tolerance to chilling injury after temperature acclimation was not attributed to the changes of abscisic acid concentration in leaves. In another experiment, sudden exposure to a photosynthetic photon flux (*PPF*) of 399 μmol·m^{-2}·s^{-1} after dark storage resulted in foliar chlorophyll degradation, restricted recovery of net CO_2 uptake rate, and severely reduced maximal quantum efficiency of the leaves. Based on these three parameters investigated, an acclimation light intensity of 140 μmol·m^{-2}·s^{-1} resulted in the best post-shipping

performance of the plants. A gradual increase from low to moderate light levels is suggested as the ideal light acclimation protocol for cultivation of mature *Phalaenopsis* plants after prolonged dark storage.

Additional key words: simulated dark shipping, chilling injury, abscisic acid, shipping temperature, net CO_2 uptake, chlorophyll fluorescence

Introduction

Phalaenopsis is an important floriculture crop. Production of potted *Phalaenopsis* is a growing industry, and it is common for plants in various stages of maturity to be exported for finishing and resale in other countries. Growers in warm climates mass-produce *Phalaenopsis* plants at high temperatures (e.g. 28-32 °C day temperatures among Taiwanese growers) to promote rapid growth during the vegetative period. Large quantities of mature plants ready for flower forcing with low temperature are exported to growers in cooler climates. *Phalaenopsis* plants exhibit crassulacean acid metabolism (CAM), hence they can tolerate shipping conditions well. The recent trend is for ready-to-force plants to be shipped by sea to save costs. Via the sea route, plants are typically shipped in total darkness at 18-20 °C for prolonged periods. For instance, it takes up to 3 weeks for *Phalaenopsis* plants from Taiwan to reach America or Europe by sea. Before and after the long-term dark shipping, the *Phalaenopsis* plants undergo at least two drastic changes, namely a drop in temperature by more than 10 °C as they are moved from the greenhouse to the shipping container, and a sharp increase in light level from total darkness during shipping to about 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ commonly used in the finishing stage of *Phalaenopsis* cultivation.

Exposure to low temperatures results in chilling injury in *Phalaenopsis*

(McConnell and Sheehan, 1978). Chilling injury symptoms in *Phalaenopsis* include water-soaked spots on leaf surfaces, yellowing of the leaves, and leaf drop. These symptoms are sometimes observed in *Phalaenopsis* plants after shipment. The protective effect of lower-temperature acclimation against chilling injury has been reported for *Phalaenopsis* plants in simulated 10-d air-freight conditions (Wang, 2007), but no detailed investigation of physiological parameters associated with chilling injury in *Phalaenopsis* plants has been reported.

A sharp increase in light level from prolonged period of total darkness to general greenhouse light levels may cause yellowing of leaves or sunburn. The lower leaves of *Phalaenopsis* are adapted to low light due to shading by upper leaves, but possess the ability to reacclimate to bright light (Lin and Hsu, 2004). The effect of light intensity on the performance of *Phalaenopsis* plants after dark storage has not been studied.

In this study, we subjected *Phalaenopsis* plants to a simulated 21-d dark shipping at different temperatures to investigate how temperature acclimation before shipping affects plant quality and investigate the relationship between different shipping temperatures and abscisic acid (ABA) concentration. In a separate experiment, the effect of various light intensities on the subsequent performance of plants after dark storage was investigated, in order to determine the optimal light intensity for *Phalaenopsis* plants just removed from storage in total darkness.

Materials and methods

Plant materials

All the experiments in this study used mature, ready-to-force *Phalaenopsis* Sogo Yukidian ‘V3’ purchased from Clone International Biotech (Pingtung, Taiwan). The

plants had 35- to 40-cm leaf span and were potted in 10.5-cm diameter, clear, soft plastic pots (0.75 L capacity) with tightly packed sphagnum moss medium. Plants were fertigated as necessary with a 20N-8.6P-16.6K fertilizer (Peters Professional 20-20-20; Scotts, Marysville, OH) at 1 $g \cdot L^{-1}$. Average day/night temperatures of 28/25 °C and a maximum photosynthetic photon flux (*PPF*) of 370 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were maintained in the greenhouse.

Simulated dark shipping conditions

Fertigation was discontinued approximately 7 days before simulated dark shipping (SDS) to reduce the risk of disease occurrence. Plants designated to receive SDS (hereafter referred to as "shipped plants") were put into cartons with shredded newspapers, and then placed in a dark growth chamber for 21 d. Unless stated otherwise, temperature was maintained at 20 °C. Relative humidity was 40% to 50% within the cartons during storage, as measured by a datalogger (H08-004-02; Onset, Buzzards Bay, MA).

Measurement of chlorophyll fluorescence and net CO_2 uptake rate

Measurements were taken on the newly matured leaf (second leaf from the apex), at the middle area near the midrib.

Leaf chlorophyll fluorescence was measured at 1300 HR with a photosynthesis yield analyzer (MINIPAM; Heinz Walz, Effeltrich, Germany). Minimal fluorescence (Fo) was determined after a 40-min dark adaptation. Maximal fluorescence (Fm) was measured after a saturation pulse. The maximal quantum efficiency Fv/Fm was calculated, where Fv = Fm – Fo.

A portable photosynthesis system (LI-6400; LI-COR, Lincoln, NE) was used to measure net CO_2 uptake rate. Because *Phalaenopsis* are CAM plants, the

measurements were done in the dark at 2100 HR in the greenhouse. Each measurement was taken on 6 cm^2 of leaf area. External air was scrubbed of CO_2 and then mixed with a supply of pure CO_2 to create a standard concentration of 350 $\mu mol \cdot mol^{-1}$. Air flow rate was controlled at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During measurement, the leaf was kept in the leaf chamber for 20 to 30 s for equilibration with the ambient microconditions. Net CO_2 uptake rate and stomatal conductance (g_S) were recorded.

Measurement of ABA concentration

Leaf discs (≈0.2 g) were collected from the second leaves from the apex. The sampling point was at the middle portion of the leaf near the midrib. The sample was frozen in liquid N_2 after fresh weight measurement. Each sample was extracted with 200 μL of 80% methanol at 25 °C for 6 h. A 100 μL aliquot of each extract was put in a 96-well plate and dried at 45 °C. The samples were redissolved and separated with reverse-phase chromatography on columns packed with C_{18}-silica material (J.T. Baker Chemicals, Philipsburg, NJ). Indirect enzyme-linked immunosorbent assay (ELISA) as described by Setter et al. (2001) was used to analyze ABA.

Measurement of leaf color and relative chlorophyll concentration

Leaf color was measured with a spectrophotometer (CM-2600d; Konica Minolta Sensing, Osaka, Japan), at the same leaf position described for chlorophyll fluorescence measurement. Leaf relative chlorophyll concentration was measured at the tip of the newly matured leaf by a chlorophyll meter (SPAD502; Minolta, Tokyo, Japan).

Expt. 1. Effects of lower-temperature acclimation before shipping and the temperature during shipment on the subsequent quality and performance of *Phalaenopsis*

Plants were grown at 30/25 °C day/night temperatures for 10 days after purchase

to mimic the growing conditions in commercial greenhouses. Plants were divided into 8 groups of 12 plants each (as shown in Table 1). Temperature acclimation was done by transferring the plants to a 25/20 °C phytotron 10 days before SDS, while non-acclimated plants were kept at 30/25 °C during that period. Both temperature-acclimated and non-acclimated groups were subjected to SDS at 25, 20, and 15 °C. At the end of SDS, the number of yellowed leaves per plant was counted. A leaf with obvious yellow coloration, or with yellowing at the base, was defined as a "yellowed leaf". The degree of chilling injury of the plants was determined using the index from 0 to 3 as described in Table 1. The concentration of ABA in newly matured leaves was measured. Chlorophyll fluorescence parameters of newly matured leaves were determined at Day 0 and Day 3 after SDS ended. The control groups were not subjected to SDS treatment and the number of yellowed leaves, the degree of chilling injury, and the leaf ABA concentration of the plants were measured at the start of the SDS period. At the end of SDS, the plants were grown at 25/20 °C day/night temperatures, and the time to spiking (i.e. inflorescence emergence) was recorded.

Expt. 2. Determination of the optimal light intensity for acclimation after a prolonged dark storage

A total of 35 plants were subjected to SDS. The plants were then placed in a growth chamber with 30/25 °C day/night temperatures and given 34, 72, 140, 200, or 399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* at a 12-h photoperiod. The light source was high-pressure sodium lamps, and the various *PPFs* were achieved by applying shade cloths and adjusting the height of benches. Control plants were not subjected to SDS and were grown in a growth chamber at 30/25 °C with a *PPF* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Net CO_2 uptake rate, g_S, and parameters of chlorophyll fluorescence were measured before storage and 0, 1, 2, 3, 4, 5, 9, 12, and 15 d after SDS was completed. Relative chlorophyll concentration and leaf color were determined 15 d after the end of storage.

After 15-day light acclimation, the plants were given 25/20 °C day/night temperatures for forcing at approximately 200-300 μmol·m^{-2}·s^{-1} *PPF*. The dates of spiking were then recorded.

Statistical analysis

Data were subjected to analysis of variance by using a completely randomized design. Means separation between treatments was determined with the least significant difference (LSD) test at $P \leq 0.05$. Statistical analyses were performed with Costat (version 6.1; CoHort Software, Monterey, CA).

Results

Expt. 1. Effects of lower-temperature acclimation before shipping and the temperature during shipment on the subsequent quality and performance of *Phalaenopsis*

Storage temperature during SDS affected the number of yellowed leaves. Plants stored at 25 °C had the highest number of yellowed leaves, while no difference in yellowing was found between plants stored at 20 and 15 °C (Table 1). There was no difference in number of yellowed leaves between acclimated and non-acclimated plants (Table 1). After storage at 15 °C, the plants suffered from chilling injury whether or not they had been acclimated, but less severe chilling injury was observed in plants with lower-temperature acclimation (Table 1). Storage temperature affected the ABA concentration in the leaves, with the highest ABA concentrations detected in plants stored at 25 °C; the ABA concentration at that temperature was similar to control plants not subjected to SDS (Table 1). Temperature acclimation of plants before storage did not affect the ABA concentration, indicating that the increased tolerance to chilling injury observed in temperature-acclimated plants shipped at 15 °C was not due to ABA accumulation (Table 1). The quality of the plants was

preserved best when stored at 20 °C.

Leaf Fv/Fm values of plants right after SDS were affected by storage temperature, where the leaves of plants stored at 25 °C had lower Fv/Fm values than the other storage temperature groups, indicating an inhibition of PS II system on plants after the dark storage at higher temperature (Table 2). Whether or not the plants had been acclimated, plants stored at 25 °C had slightly higher Fo but significantly lower Fm than the other groups, indicating that the decrease in Fv/Fm at 25 °C was caused by lower Fm values (Table 2). There was no difference in leaf chlorophyll fluorescence parameters of all plants 3 days after SDS was ended (Table 2), demonstrating that the inhibition of PS II in *Phalaenopsis* due to 21-d dark storage at 25 °C was temporary and reversible.

Temperature-acclimated plants required shorter forcing period after SDS before spiking; for example, after 21-d storage at 20 °C, acclimated plants required 18.0 days to spiking on average, compared with 24.5 days for non-acclimated plants (data not shown).

Expt. 2. Determination of the optimal light intensity for acclimation after a prolonged dark storage

The effect of different light levels during the recovery period after 21-d SDS was determined. Low net CO_2 uptake rate was observed in all shipped plants at the end of the SDS (Fig. 1A). One day after SDS was ended, various levels of *PPF* produced a small increase in net CO_2 uptake rate, and the values in the different treatments were similar. Under 140 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*, net CO_2 uptake rate of the plants increased rapidly to the level of control plants within 4 d after SDS was ended (Fig. 1A). Under 34 and 72 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*, net CO_2 uptake rate of the plants increased with time in the early recovery period, but soon tapered off after 4 to 5 d and did not

reach the level of control plants within 15 d after the end of SDS (Fig. 1A). The recovery of CO_2 uptake was greatly inhibited by a high light intensity (399 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and increased slowly with time during the recovery period; even after 15-d exposure to light, the net CO_2 uptake rate was just a little more than half the value measured in control plants (Fig. 1A). The g_S exhibited a similar trend to that of net CO_2 uptake by and large (Fig 1B). The relationship between *PPF* and net CO_2 uptake rate changed with time after SDS (Fig. 2). There was little correlation between *PPF* and net CO_2 uptake rates on Day 1 ($R^2 = 0.23$, $P = 0.02$) and the net CO_2 uptake rates were similarly low in all shipped plants regardless of light intensity given. On Day 2, net CO_2 uptake rate of shipped plants increased markedly except for those given 399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* (Fig. 2). Net CO_2 uptake rates and *PPF* were found to be quadratically correlated on Days 2 through 9, and the curve maxima shifted to the direction of increasing *PPF* with time after storage, indicating that there is a gradual increase over time in the light requirement of *Phalaenopsis* plants after a dark storage (Fig. 2). However, a *PPF* value of 399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ always corresponded to a sharp decline of net CO_2 uptake on the curves (Fig. 2). These results showed that net CO_2 uptake of shipped plants was able to recover quickly to a normal level at a medium light intensity (140-200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*), but was restricted at low (34-72 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*) and high (399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*) light intensities.

Just after SDS was ended, the maximal quantum efficiency (Fv/Fm) of shipped plants were similar to that of control, indicating that PS II of plants was not damaged during storage (Table 3). After exposure to light, all treatments showed a decrease in Fv/Fm in varying degrees by Day 1 (Table 2), where the largest drop was found under 399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* and less significant on 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* (Table 3). Lower light intensities resulted in very slight decrease in Fv/Fm, and the values were still comparable to that of control plants (Table 3). By Day 3 through 12, the Fv/Fm levels of shipped plants acclimated in lower light intensities (34-140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*)

became stabilized at values close to that of control plants, while the values were slightly and significantly lower, respectively, for plants under 200 and 399 μmol·m^{-2}·s^{-1} *PPF* (Table 3).

After 15-d light acclimation, the shipped plants showed decreased relative chlorophyll concentration as represented with SPAD values, and increased L, a, and b values as the light intensity given to the plants was increased (Fig. 3). Higher L, a, and b values represented lighter color, less green coloration, and more yellow coloration, respectively. The leaf relative chlorophyll concentration of plants given 399 μmol·m^{-2}·s^{-1} *PPF* was significantly lower than that of control plants, while that of plants given 200 μmol·m^{-2}·s^{-1} *PPF* were only moderately lower compared with control (Fig. 3A). The L, a, and b values of plants at 399 μmol·m^{-2}·s^{-1} *PPF* indicated significant lightening of green color and more pronounced yellow hue, while the leaf color parameters of plants given 140 μmol·m^{-2}·s^{-1} and 200 μmol·m^{-2}·s^{-1} *PPF* were comparable to control (Fig. 3B to D). Deeper green hue in the leaves was detected in plants given 34 and 72 μmol·m^{-2}·s^{-1} *PPF* (Fig. 3B to D).

Among the shipped plants, those acclimated at 140 μmol·m^{-2}·s^{-1} *PPF* produced the best spiking quality, i.e. similar timing and percentage of spiking compared with control plants, with 100% spiking achieved by Day 60 after forcing was started (data not shown). At the two lowest light intensities during acclimation, some early spiking was observed by Day 20, but lower spiking percentages was observed on Day 60 (data not shown). Acclimation at *PPFs* of 34, 72 and 399 μmol·m^{-2}·s^{-1} resulted in only 71% spiking by Day 60; at the same day, the observed the percentage of spiking at 200 μmol·m^{-2}·s^{-1} *PPF* was 86% (data not shown). Shipped plants acclimated at 399 μmol·m^{-2}·s^{-1} *PPF* had the most non-uniform spiking overall, with only half the spiking percentage of control at Day 40 (data not shown).

Table 1. Effects of thermal acclimation at 25/20 °C for 10 days, and temperature of simulated dark shipping on subsequent leaf condition, degree of chilling injury, and abscisic acid (ABA) concentration of *Phalaenopsis* Sogo Yukidian 'V3'. Treated plants were stored in a cooler for 21 days (n = 12).

Storage temp. (°C)	Temp. acclimation	Before simulated dark shipping			The end of simulated dark shipping		
		Yellowed leaf (no.)	Degree of chilling injury[y]	ABA concn. (pmol·g^{-1}FW)	Yellowed leaf (no.)	Degree of chilling injury	ABA concn. (pmol·g^{-1}FW)
None[z]	No	0.0	0.0	10.0			
None	Yes	0.0	0.0	9.6			
25	No				0.6	0.0	10.4
	Yes				0.6	0.0	10.4
20	No				0.1	0.1	9.8
	Yes				0.0	0.0	7.8
15	No				0.2	2.1	8.2
	Yes				0.1	1.3	7.2
Significance							
Storage temp.		-	-	-	**	***	*
Temp. acclimation		NS	NS	NS	NS	*	NS
Temp. acclimation X Storage temp.		-	-	-	NS	*	NS

[z] Data were collected on plants grown in greenhouse with day/night temperatures of 30/25 °C before the simulated dark shipping.
[y] 0 = no chilling injury; 1 = slight water-soaked spots on leaf surface, reversible after subsequent growth; 2 = one leaf had a yellowed leaf base; 3 = two or more leaves had obvious yellow spots, which covered more than 20% of the leaf areas.
NS,*,**,*** Nonsignificant or significant at $P \leqq 0.05$, 0.01, or 0.001, respectively.

Table 2. Effects of thermal acclimation at 25/20 °C for 10 days, and temperature of simulated dark shipping on subsequent chlorophyll fluorescence parameters of *Phalaenopsis* Sogo Yukidian 'V3'. Treated plants were stored in a cooler for 21 days (n = 6).

Storage temp. (°C)	Temp. acclimation	Before simulated dark shipping			The end of simulated dark shipping			3 days after the end of simulated dark shipping		
		Fv/Fm	Fo	Fm	Fv/Fm	Fo	Fm	Fv/Fm	Fo	Fm
None[z]	No	0.82	352	2008						
None	Yes	0.83	352	2026						
25	No				0.77	314	1346	0.78	328	1495
	Yes				0.74	325	1256	0.79	321	1533
20	No				0.79	298	1432	0.77	334	1480
	Yes				0.80	302	1544	0.77	335	1472
15	No				0.80	308	1564	0.78	331	1518
	Yes				0.79	322	1540	0.78	321	1488
Significance										
Storage temp.		-	-	-	***	*	***	NS	NS	NS
Temp. acclimation		NS	NS	NS	NS	NS	NS	NS	NS	NS
Temp. acclimation X Storage temp.		-	-	-	NS	NS	*	NS	NS	NS

[z] Data were collected on plants grown in a greenhouse with day/night temperatures of 30/25 °C before the simulated dark shipping.
NS,*,*** Nonsignificant or significant at $P \leqq 0.05$, or 0.001, respectively.

Table 3. Changes of maximal quantum efficiency (Fv/Fm) in *Phalaenopsis* Sogo Yukidian 'V3' 0, 1, 3, 5, 9, and 12 d after a 21-d simulated dark shipping at 20 °C. Various levels of photosynthetic photon flux (*PPF*) as indicated were provided after the simulated dark shipping. (Hou and Chang, 2010)

PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Time after the end of simulated dark shipping (d)					
	Day 0	Day 1	Day 3	Day 5	Day 9	Day 12
Control[z]	0.79 b[y]A[x]	0.79 a A	0.79 a A	0.78 ab A	0.78 ab A	0.77 a A
399	0.81 a A	0.62 c B	0.39 c C	0.41 c C	0.31 c C	0.38 b C
200	0.81 a A	0.71 b B	0.72 b B	0.72 b B	0.73 b B	0.74 a B
140	0.81 a A	0.76 a C	0.77 ab BC	0.77 ab BC	0.79 ab B	0.78 a B
72	0.82 a A	0.79 a B	0.81 a A	0.82 a A	0.82 a A	0.82 a A
34	0.81 a AB	0.80 a B	0.81 a A	0.82 a A	0.81 a A	0.82 a A

[z] Plants were not subjected to simulated dark shipping and grown in a growth chamber with day/night temperature of 30/25 °C and with a *PPF* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

[y] Means followed by a different lower case letter in columns are significantly different at $P \leq 0.05$ by the least significant difference (LSD) test (n=7).

[x] Means followed by a different upper case letter in rows are significantly different at $P \leq 0.05$ by the LSD test.

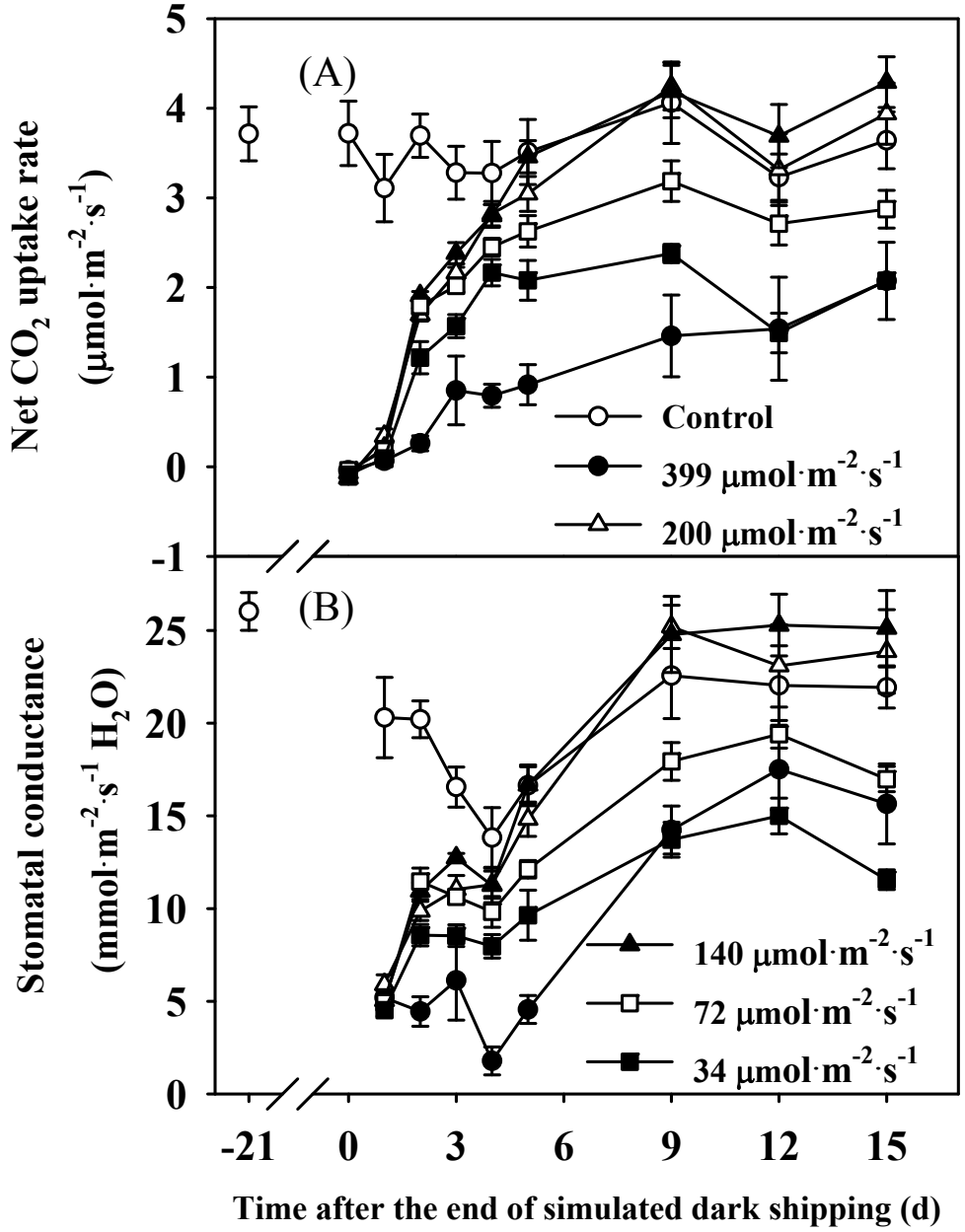

Fig. 1. Effect of light acclimation at various levels of photosynthetic photon flux (*PPF*) on net CO_2 uptake rate (A) and stomatal conductance (B) in *Phalaenopsis* Sogo Yukidian 'V3'. Measurements were taken before and 0, 1, 2, 3, 4, 5, 9, 12, and 15 d after a 21-d simulated dark shipping at 20 °C. Controls did not receive simulated shipping and were grown in a growth chamber with day/night temperatures of 30/25 °C and with a *PPF* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. All shipped plants were moved to the same growth chamber as controls but received various *PPF* as indicated after the simulated dark shipping. Bars indicate SE of the means (n=7). (Hou et al., 2010)

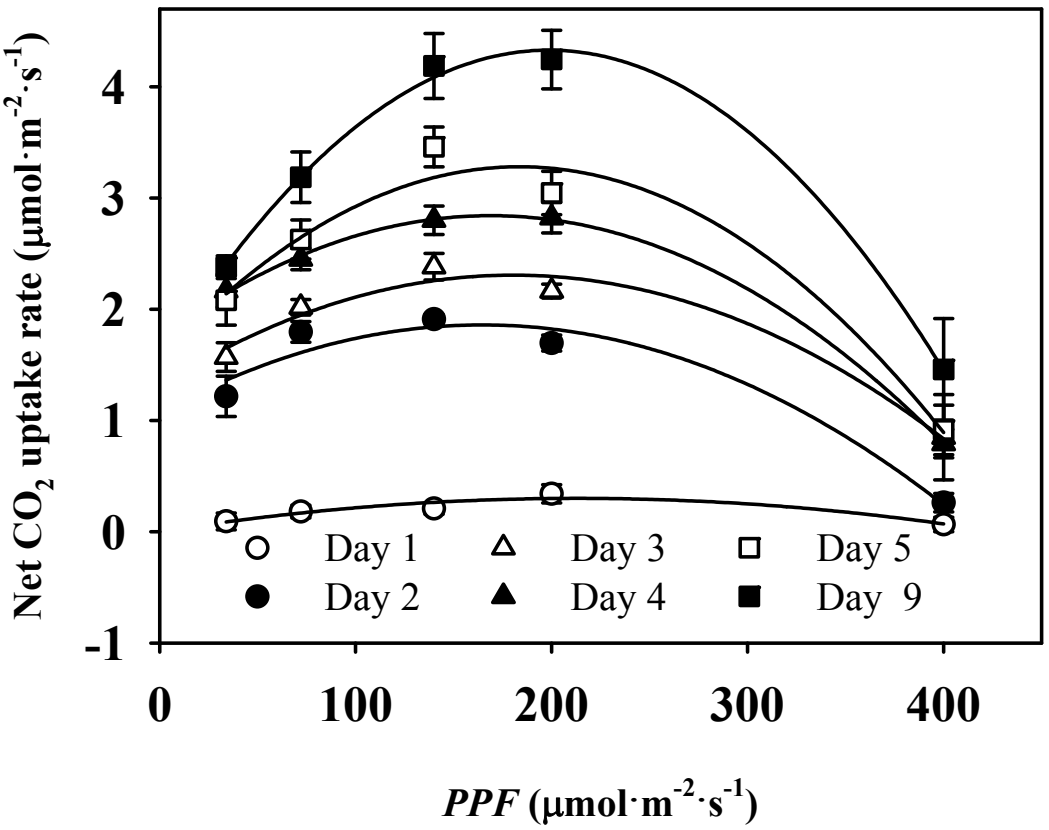

Fig. 2. The relationship between photosynthetic photon flux (*PPF*) and net CO_2 uptake rate of *Phalaenopsis* Sogo Yukidian 'V3' on Day 1 (-○-, y=0.0002 + 0.0028x-0.0000066x^2, R^2=0.23, *P*=0.02), Day 2 (-●-, y=1.07+0.0096x-0.0000292x^2, R^2=0.81, *P*<0.0001), Day 3(-△-, y=1.31+0.011x-0.0000305x^2, R^2=0.55, *P*<0.0001), Day 4 (-▲-, y=1.75 + 0.013x-0.0000.83x^2, R^2=0.85, *P*<0.0001), Day 5 (-□-, y=1.56 + 0.019x-0.000051x^2, R^2=0.74, *P*<0.0001), and Day 9 (-■-, y=1.52 +0.028 x -0.000071x^2, R^2=0.69, *P*<0.0001) from the completion of simulated dark shipping. Bars indicate SE of the means (n=7). (Hou et al., 2010)

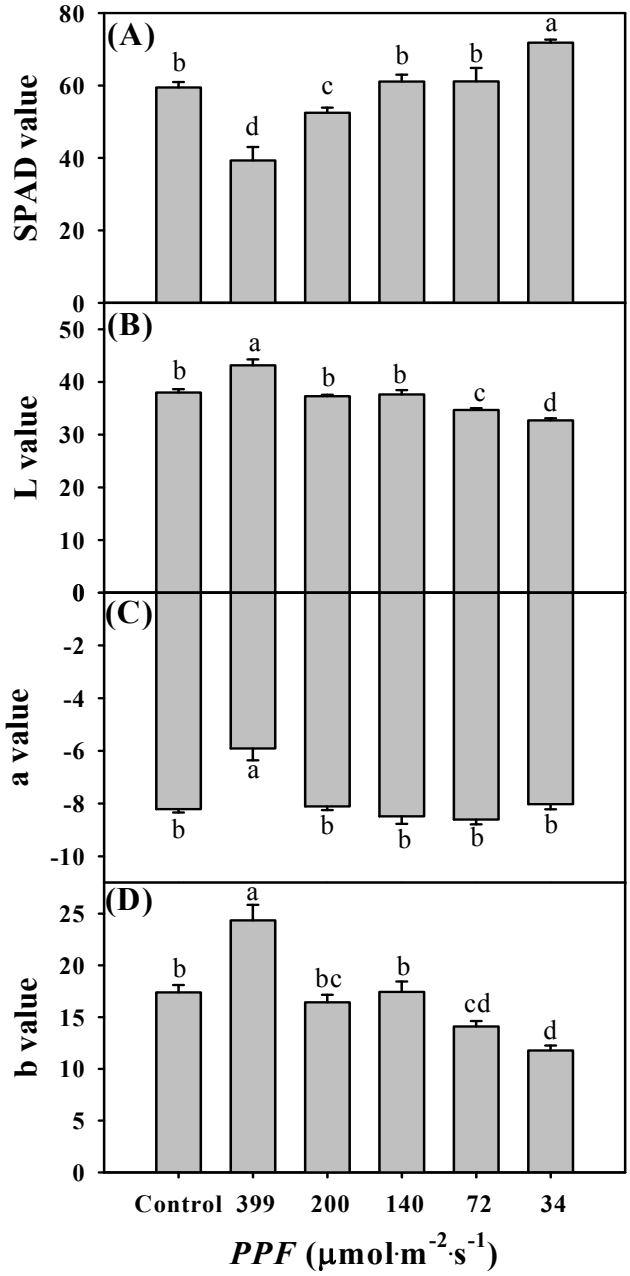

Fig. 3. Effect of 15-d light acclimation with various photosynthetic photon flux (*PPF*) on relative leaf chlorophyll concentration and leaf color of *Phalaenopsis* Sogo Yukidian 'V3' after a 21-d simulated dark shipping, as measured by SPAD and Lab values, respectively. Controls were not subjected to simulated shipping and were grown in a growth chamber with day/night temperatures of 30/25 °C and with a *PPF* of 200 μmol·m^{-2}·s^{-1}. Shipped plants were placed in the same growth chamber as controls but received various *PPF*s as indicated after the simulated dark shipping. (Hou and Chang, 2010)

Discussion

The shipping temperature of *Phalaenopsis* plants by sea route is set between 18-20 °C on average. A storage temperature of 20 °C was found to preserve the quality of the plants well during simulated shipment, whereby none to very slight chilling injury was observed on both temperature-acclimated and non-acclimated plants (Table 1). However, incidence of chilling injury was significant at 15 °C, and plants acclimated to lower temperature before shipment could better withstand the effects of chilling (Table 1). Therefore, temperature acclimation of *Phalaenopsis* plants before shipment is highly recommended, because different clones may have different susceptibility to chilling injury, and plants may be subjected to greater temperature drop in practice if they were grown in warmer greenhouses. On the other hand, we found that a shipping temperature of 25 °C had detrimental effects on the shipped plants (Tables 1 and 2).

Prolonged dark shipping at 20 °C did not affect PS II in *Phalaenopsis* plants as indicated by Fv/Fm value comparable to the level found in control plants (Tables 2 and 3). However, different light intensities during the recovery period after shipping were found to significantly affect net CO_2 uptake rate, Fv/Fm, and relative chlorophyll concentration of newly matured leaves. After 21 d of total darkness, a sudden exposure to high light levels commonly used for growing *Phalaenopsis* in the finishing stage (399 $\mu mol \cdot m^{-2} \cdot s^{-1}$) produced symptoms of sunburn, and decreased the Fv/Fm and net CO_2 uptake rate of the leaves (Table 3, Figs. 1-3). Therefore, acclimation at low to moderate light levels is necessary to enhance the performance of shipped plants. Effects of low to moderate light levels during the acclimation period were compared, and continuous low light levels did not damage the plants but resulted in restricted net CO_2 uptake. Shipped plants given moderate light levels of 140 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* recovered the uptake of CO_2 quickly to normal levels (Fig. 1),

but a direct exposure to 200 μmol·m^{-2}·s^{-1} *PPF* still caused slight damaging effects on the leaves (Table 3, Fig. 3). Further analysis of the data showed that the light requirement of *Phalaenopsis* plants after prolonged dark shipping gradually increased with time (Fig. 2). Hence a gradual increase from low to moderate light level is recommended for 6 to 9 d after prolonged dark shipping. However, if gradual adjustment of light levels over a few days is not feasible, a direct acclimation at 140 μmol·m^{-2}·s^{-1} *PPF* is acceptable.

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